Polydatin attenuates cardiac hypertrophy through modulation of cardiac Ca\(^{2+}\) handling and calcineurin-NFAT signaling pathway

Wenwen Ding,1,2* Ming Dong,1* Jianxin Deng,2 Dewen Yan,3 Yun Liu,1 Teng Xu,1 and Jie Liu1,2
1Department of Pathophysiology, School of Medicine, Shenzhen University, Shenzhen, China; 2Department of Pathophysiology, Southern Medical University, Guangzhou, China; and 3Department of Endocrinology, The First Affiliated Hospital of Shenzhen University, Shenzhen, China

Submitted 14 January 2014; accepted in final form 20 June 2014

Ding W, Dong M, Deng J, Yan D, Liu Y, Xu T, Liu J. Polydatin attenuates cardiac hypertrophy through modulation of cardiac Ca\(^{2+}\) handling and calcineurin-NFAT signaling pathway. Am J Physiol Heart Circ Physiol 307: H792–H802, 2014. First published July 11, 2014; doi:10.1152/ajpheart.00017.2014.—Polydatin (PD), a resveratrol glucoside extracted from the perennial herbage Polygonum cuspidatum, has been suggested to have wide cardioprotective effects. This study aimed to explore the direct antihypertrophic role of PD in cultured neonatal rat ventricular myocytes (NRVMs) and its therapeutic effects against pressure overload (PO)-induced hypertrophic remodeling and heart failure. Furthermore, we investigated the mechanisms underlying the actions of PD. Treatment of NRVMs with phenylephrine for 72 h induced myocyte hypertrophy, where the cell surface area and protein levels of atrial natriuretic peptide and \(\beta\)-myosin heavy chain (\(\beta\)-MHC) were significantly increased. The amplitude of systolic Ca\(^{2+}\) transient was increased, and sarcoplasmic reticulum Ca\(^{2+}\) recycling was prolonged. Concomitantly, calcineurin activity was increased and NFAT protein was imported into the nucleus. PD treatment restored Ca\(^{2+}\) handling and inhibited calcineurin-NFAT signaling, thus attenuating the hypertrophic remodeling in NRVMs. PO-induced cardiac hypertrophy was produced by transverse aortic constriction (TAC) in C57BL/6 mice, where the left ventricular posterior wall thickness and heart-to-body weight ratio were significantly increased. The cardiac function was increased at 5 wk of TAC, but significantly decreased at 13 wk of TAC. The amplitude of Ca\(^{2+}\) transient and calcineurin activity were increased at 5 wk of TAC. PD treatment largely abolished TAC-induced hypertrophic remodeling by inhibiting the Ca\(^{2+}\)-calcineurin pathway. Surprisingly, PD did not inhibit myocyte contractility despite that the amplitude of Ca\(^{2+}\) transient was decreased. The cardiac function remained intact at 13 wk of TAC. In conclusion, PD is beneficial against PO-induced cardiac hypertrophy and heart failure largely through inhibiting the Ca\(^{2+}\)-calcineurin pathway without compromising cardiac contractility.

polydatin; hypertrophy; heart failure; calcium transient; calcineurin; NFAT; transverse aortic constriction

Cardiac hypertrophy is an adaptive response most commonly to hypertension and an independent risk factor for the development of heart failure and more generally an increased morbidity and mortality (1, 18). At the cellular level, enlargement of the cardiomyocyte involves multiple events, including gene transcription and protein translation/synthesis, which are regulated by multiple signaling cascades. Among them, enhancement of calcium-regulated signaling pathways plays a major role in the development of pathological cardiac hypertrophy (15, 26, 33). Accordingly, manipulation of cardiac Ca\(^{2+}\) signaling represents a logical approach to treatment of cardiac hypertrophy.

Cardiac Ca\(^{2+}\) signaling is a delicate interplay of multiple Ca\(^{2+}\) handling proteins (2, 3, 12, 31). At systole, the activation of L-type Ca\(^{2+}\) channel (LTCC) allows extracellular Ca\(^{2+}\) influx into cardiomyocytes upon membrane depolarization, triggering sarcoplasmic reticulum (SR) Ca\(^{2+}\) release via Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) mechanism. At diastole, the increased intracellular Ca\(^{2+}\) is sequestered into the SR by the SR Ca\(^{2+}\)-ATPase (SERCA) and extruded from cytoplasm by the sodium/calcium (Na\(^{+}/\)Ca\(^{2+}\)) exchanger and sarcolemmal Ca\(^{2+}\)-ATPase. The activity of LTCC can be inhibited by Ca\(^{2+}\)-channel blockers, which have been confirmed to be therapeutically effective for the treatment of cardiac hypertrophy in many animal models (14, 29). However, clinical blockade of LTCC has dubious benefit on cardiovascular mortality, despite that calcium channel antagonists induce regression of hypertrophy (19, 37). Given the essential role of intracellular Ca\(^{2+}\) in cardiac contraction, blockade of LTCC would decrease cardiac contractile function. Furthermore, blockade of noncardiac Ca\(^{2+}\) channels induces side effects, including systemic hypotension, constipation, edema, etc. (7, 16). All these shortcomings of LTCC antagonists counteract their beneficial effects on the treatment of cardiac hypertrophy. Thus new agents (drugs) that can suppress cardiac Ca\(^{2+}\) signaling without causing the side effects are highly expected.

Polydatin (PD), a resveratrol glucoside with a 3,4,5-trihydroxystibien-3-\(\beta\)-d-mono-D-glucoside molecular structure, is a natural component extracted from the perennial herbage Polygonum cuspidatum Sieb. et Zucc (5, 10). So far, there is only one study reporting that PD attenuated ventricular remodeling in vivo by inhibiting the activation of neurohormone (13). Whether PD has a direct antihypertrophic effect and whether it modifies the heart failure process in vivo remain unknown. Recently, we found that PD modulated Ca\(^{2+}\) handling and cardiac contractility. When acutely applied to isolated rat ventricular myocytes, PD significantly decreased LTCC current (\(I_{\text{Ca,L}}\)) and moderately decreased systolic Ca\(^{2+}\) transient. Interestingly, PD slightly increased rather than decreasing cardiac contractility (8). The findings in normal cardiomyocytes lead us to the hypothesis that PD suppresses hypertrophic stimuli-induced upregulation of Ca\(^{2+}\) signaling and thus exerts a direct antihypertrophic effect, without compromising cardiac contractile function. If the hypothesis is true, combining with previous findings that PD did not reduce blood pressure and interfere with systemic hemodynamics in normal animals (30, 38), it is rational to speculate that PD is a good candidate drug for the treatment of cardiac hypertrophy.
To test this hypothesis, we explored the therapeutic effect of PD on attenuation of hypertrophy in cultured neonatal rat ventricular myocytes stimulated with phenylephrine (PE) and in a pressure overload (PO)-induced hypertrophic mouse model. Furthermore, we investigated the effects of PD on cardiac Ca\(^{2+}\) handling and contractility, as well as the Ca\(^{2+}\)/calmodulin-activated calcineurin-nuclear factor of activated T cells (NFAT) signaling pathway, which has been shown to play a major role in the development of pathological cardiac hypertrophy (6, 24, 25, 34). Here, we demonstrated that PD inhibited the development of cardiac hypertrophy in vitro as well as in vivo by inhibiting the Ca\(^{2+}\) signal and calcineurin-NFAT signaling pathway.

**METHODS**

**Animals.** Animals were purchased from the Animal Center of Southern Medical University and handled according to a protocol approved by the Institutional Care and Use Committee of Shenzhen University that conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85–23, revised 1996).

**Culture of neonatal rat ventricular myocytes (NRVMs) and treatment protocol.** Neonatal rat ventricular myocytes (NRVMs) were isolated from 2-day-old Sprague-Dawley rats and cultured as previously described (23). In brief, hearts were obtained following decapitation and immersed in PBS and minced with scissors. The small pieces of heart tissue were digested with 0.25% trypsin-EDTA in PBS at 37°C. The isolated cells were put in fetal bovine serum (FBS) and pelleted by centrifugation at 1,000 rpm for 5 min. The pelleted cells were resuspended in DMEM containing 10% FBS, 1% penicillin-streptomycin, and then preplated for 30 min at 37°C to allow fibroblasts to adhere to the plate. The unadhered cells were pelleted again and resuspended in DMEM containing 10% FBS, 1% penicillin-streptomycin, and bromodeoxyuridine (1:100, to inhibit fibroblast growth), which were finally plated at a concentration of about 1 million cells per 35-mm plate. To produce hypertrophy, NRVMs were treated with 20 μM phenylephrine (PE, Sigma) for 72 h after 24 h of serum starvation in the absence or presence of PD (25–75 μM). PD with a purity of 98.87% was kindly provided by Haiwang (Shenzhen, Guangdong, China).

**Measurement of cell surface area.** The surface area of a single myocyte was measured with Image-Pro Plus Data Analysis Program (Media Cybernetics, Silver Spring, MD). Quantification of cell surface area was performed by measuring 50 random cells from three experiments, and the average value was used for analysis.

**Calcineurin enzymatic assay.** Calcineurin activity was determined in myocyte extracts using a colorimetric Calcineurin Cellular Activity Assay Kit following the manufacturer’s instructions (Enzo Life Sciences) (21). Calcineurin activity was determined as nanomoles phosphate released at 620 nm using a SpectraMax 5 (Molecular Devices, Sunnyvale, CA) plate reader.

**Western blotting.** Cell lysates were resolved in SDS polyacrylamide gel electrophoresis (PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore). Nuclear protein extraction...
was performed using a Nuclear and Cytoplasmic Protein Extraction kit according to the manufacturer’s instruction (Pierce, Thermo Scientific). Ventricular tissue proteins were obtained by homogenizing with lysis buffer. Target proteins were reacted with their respective antibodies, atrial natriuretic peptide (ANP; Santa Cruz Biotechnology), β-MHC (Santa Cruz Biotechnology), calcineurin (Abcam) (28), LaminA/C (Cell Signaling Technology), NFAT3 (Cell Signaling Technology), α-AMPK (Cell Signaling Technology), p-α-AMPK (Cell Signaling Technology), and then incubated with respective secondary antibody. β-Actin (ZSGB-Bio, China) or GAPDH (Santa Cruz Biotechnology).

Fig. 2. Comparison of the antihypertrophic effects of PD and trans-resveratrol in cardiac myocytes. A: molecular structures of PD, trans-resveratrol, and cis-resveratrol. B and C: representative images (B) of cultured NRVM and statistics (C) of the cell surface area of NRVMs in control (C) and PE-stimulated cells (PE) with or without PD or trans-resveratrol (RV) treatment. D: representative images and quantification of the protein levels of ANP. E: representative images and quantification of mRNA levels of β-MHC. F: representative images and quantification of the protein levels of β-MHC. *P < 0.05, **P < 0.01 vs. control (C); #P < 0.05, ##P < 0.01 vs. PE.
Biotechnology) was used as a loading control for ANP and calcineurin proteins, and LaminA/C as a loading control for NFAT3 nuclear protein.

**Determination of β-MHC mRNA level.** Total RNA was extracted using TRIZOL (Invitrogen) according to the manufacturer’s instructions. The mRNA level of β-MHC was determined with reverse transcription-polymerase chain reaction (RT-PCR) with GAPDH as a housekeeping gene. The PCR conditions were as follows: denaturation at 94°C for 30 s, followed by annealing at 57°C for 30 s and elongation at 72°C for 1 min. Both genes were amplified for 35 cycles. The sequences of β-MHC primers were forward 5'-TCGTGGAGCGCGCAACAAC-3' and reverse 5'-TCAAAGGCTCCAGGTCTCAGGGCT-3'. The sequences of GAPDH primers were forward 5'-GCATGTCAGATCCACAACGG-3' and reverse 5'-GCATGTCAATCCACACACGG-3'.

**Transverse aortic constriction (TAC).** C57BL/6 male mice (9–10 wk, 18–22 g) were randomly divided into four groups: sham and TAC operated with or without PD treatment, 10 in each group. The TAC model was produced as described previously (22). In brief, the animal was endotracheally intubated under anesthesia with a mixture of pentobarbital sodium (50 mg/kg ip). The chest cavity was entered in the second intercostal space at the left upper sternal border, and the heart was removed from the chest and cleaned and flushed with a Ca²⁺-free buffer containing (in mM) 120 NaCl, 5.4 KCl, 1.2 MgSO₄, 1.2 NaH₂PO₄, 5.6 glucose, 20 NaHCO₃, 10 2,3-butanedione monoxime (BDM; Sigma), and 5 taurine, 10 HEPES (pH = 7.4), and perfused using a Langendorff apparatus. All solutions were bubbled with 100% O₂. The enzymatic digestion was initiated by adding collagenase type B (0.75 mg/ml; Worthington) and protease type XIV (0.02 mg/ml; Sigma) to the perfusion solution. When the heart became swollen and hard after 3 min of digestion, 50 μM Ca²⁺ was added to the enzyme solution and perfused for about 30 min. Following the perfusion procedure, the heart was minced into small chunks, and single cells were shaken loose from the heart tissue and stored in HEPES-buffered solution containing (mM) 1 CaCl₂, 137 NaCl, 5.4 KCl, 15 dextrose, 1.3 MgSO₄, 1.2 NaH₂PO₄, and 20 HEPES, adjusted to pH 7.4 with NaOH. Cells were used for the following experiments within 4 h after isolation.

**Ca²⁺ transient detection and contraction measurement.** Cells loaded with Ca²⁺ indicator fluo-4 AM (5 mmol/l, for 8 min) (Invitrogen) were placed in a recording chamber. Confocal line-scan imaging was carried out in cells at 488 nm excitation and 505 nm collection with a Zeiss 710 inverted confocal microscope (Carl Zeiss, Oberkochen, Germany) with 40× oil immersion lens (NA 1.3). After the cells were stimulated with field stimulation (1 Hz) to reach a steady state, confocal line-scan imaging was acquired at a sampling rate of 3.84 ms per line. Myocyte contraction was measured by detecting the length of two edges of the cell along with the time of

**Fig. 3.** PD restored abnormal Ca²⁺ signaling induced by PE stimulation. A: representative confocal line-scan images of Ca²⁺ transient along with their spatial averages in response to field stimulation (1 Hz). B–D: statistics of the amplitude (ΔF/FO, B), half-time of decay (T½, C), and rise time (D) of Ca²⁺ transients. *p < 0.05, **p < 0.01 vs. control; #p < 0.05, ##p < 0.01 vs. PE (n = 72–85 in each group).
stimulation. Myocytes were superfused with HEPES-buffered external solution during the experiment.

Statistical analysis. All values were expressed as means ± SE. Statistical analysis was performed by one-way analysis of variance (ANOVA) for multiple comparisons, followed by the Dunnett’s test to evaluate the difference between two groups through the software of SPSS version 13.0. Values of $P < 0.05$ were considered statistically significant.

Fig. 4. Effects of PD on calcineurin activity and nuclear NFAT protein level. A: representative Western blots and quantification of the protein levels of calcineurin (CaN) in control and PE-treated cells with or without PD treatment. B: measurement of CaN activity in 4 groups. CaN activity was normalized by CaN protein in each group. C and D: representative Western blots and quantification of the protein levels of nuclear NFAT3 (n-NFAT3, C) and cytosolic NFAT3 (c-NFAT3, D) in 4 groups. E: representative images of NRVMs and statistics of the cell surface area in control (C) and PE-stimulated cells (PE) with or without PD and nifedipine (Nife) or FK506 (FK) treatment. F: representative Western blots and quantification of the protein levels of ANP in 6 groups. *$P < 0.05$, **$P < 0.01$ vs. control; #$P < 0.05$, ##$P < 0.01$ vs. PE ($n = 4$ in each group).
RESULTS

PD inhibited phenylephrine (PE)-induced cardiomyocyte hypertrophy. The in vitro cardiomyocyte hypertrophic model was successfully produced by treating the cultured neonatal rat ventricular myocytes (NRVMs) with 20 μM PE for 3 days, in which the cell surface area was significantly increased by 56.5 ± 6.2% (2,089 μm²) in PE-treated cells vs. 1,334 μm² in control, *P < 0.01, Fig. 1A), and the protein levels of ANP and β-MHC were remarkably increased (Fig. 1, B and C). PD treatment alone had no significant effects on the hypertrophic parameters observed. However, PD treatment (20–75 μM) dose-dependently reduced PE-induced hypertrophic remodeling. PD at the concentration of 50 μM caused significant inhibition of PE-induced enlargement of cell size (Fig. 1A). In parallel, the increased protein levels of ANP and β-MHC were largely reduced by 50 μM PD treatment (Fig. 1, B and C).

PD is an analog of trans-resveratrol. Figure 2A demonstrates the molecular structures of PD, trans-resveratrol and cis-resveratrol. Previous studies have reported that resveratrol can significantly inhibit myocyte hypertrophy (9, 17). We thus compared the effects of PD and trans-resveratrol (RV) on the hypertrophic parameters, including cell surface area and protein levels of ANP and β-MHC, in PE-stimulated NRVMs and found that the effect of PD (50 μM) on suppression of myocardial hypertrophy was comparable to that of RV (50 μM, Fig. 2, B–F).

Effects of PD on PE modulation of cardiac Ca²⁺ signaling. Although a previous study demonstrated PD acutely modulated Ca²⁺ handling in isolated adult rat cardiomyocytes (8), it remains unknown how PD regulates the abnormal Ca²⁺ handling induced by pathological PE stimulation. We thus investigated action potential (AP)-elicited Ca²⁺ transient in PE-stimulated cells with or without PD treatment. As shown in Fig. 3, A and B, PE stimulation significantly increased the amplitude of AP-elicited Ca²⁺ transient indexed by ΔF/F₀. In contrast to accelerating SR Ca²⁺ recycling by acute application of PE, chronic application of PE (for 72 h) slowed SR Ca²⁺ recycling, where the half time decay (T₁/₂) of Ca²⁺ transient was prolonged (Fig. 3C). The result indicates that the SERCA function was impaired. Moreover, sustained PE stimulation increased the rise time of Ca²⁺ transient, suggesting the synchrony of intracellular Ca²⁺ release was impaired (Fig. 3D). Consistent with the previous study, PD moderately decreased the amplitude of Ca²⁺ transient in normal cells by 12.4%. However, PD treatment (50 μM) significantly decreased the amplitude of Ca²⁺ transient which was increased by PE stimulation (by 20.7%, Fig. 3, A and B). Furthermore, PD treatment normalized the rise time and T₁/₂ of Ca²⁺ transient prolonged by PE stimulation, indicating the restoration of the synchrony of intracellular Ca²⁺ release and SR Ca²⁺ recycling (Fig. 3, C and D). The data collectively indicate that PD corrects abnormal Ca²⁺ handling induced by long-term PE stimulation.

PD suppressed PE-activated calcineurin-NFAT signaling pathway. It is well established that an increase in intracellular Ca²⁺ activates calcineurin-NFAT signaling pathway, which plays a major role in the pathogenesis of cardiac hypertrophy. The above finding suggests that PE activates this hypertrophic pathway and PD may inhibit it. As anticipated, PE significantly increased calcineurin activity by 60.8 ± 7.2% (*P < 0.05) without significantly changing the protein expression (*P > 0.05, Fig. 4, A and B). Concomitantly, NFAT transferred into nuclear, whereby the nuclear NFAT protein level was significantly increased by 50.3 ± 4.6% (*P < 0.01) with PE stimulation (Fig. 4C), but cytosolic protein level of NFAT was significantly decreased (Fig. 4D). PD treatment (50 μM) had no significant effect on calcineurin activity and NFAT nuclear protein level in control cells. In contrast, PD significantly reduced PE-induced upregulation of calcineurin activity and NFAT nuclear import (Fig. 4, B and C). The results implicate that PD inhibits the activation of the calcineurin-NFAT signaling pathway, contributing to PE-induced myocyte hypertrophy.

To further confirm that PD attenuated myocyte hypertrophy through inhibition of the Ca²⁺-calcineurin signaling pathway, we cotreated PE-stimulated cells with PD and nifedipine (20 μM), the LTCC blocker, or FK506 (200 nM), the calcineurin inhibitor. The results show that nifedipine and FK506 treatment alone significantly inhibited PE-induced increase of cell size and ANP protein level (Fig. 4, E and F), suggesting the important role of the Ca²⁺-activated calcineurin pathway in the pathogenesis of hypertrophic remodeling. Cotreating PE-stimulated cells with PD and nifedipine or FK506 only slightly increased the effect of PD on cell size and ANP protein level (Fig. 4, E and F). The results further indicate the causal relationship between inhibition of Ca²⁺-calcineurin pathway and attenuation of myocyte hypertrophy by PD treatment.

It has been suggested that the NFAT pathway can be regulated by AMP-activated protein kinase (AMPK) (11, 20). A previous study has demonstrated that resveratrol exerts antihypertrophic effects by activating AMPK, thus suppressing the NFAT pathway and protein synthesis and gene transcription (4). To test whether this is the case for PD’s action, we examined the levels of phosphorylated AMPK. We examined the effect of PD on the level of phosphorylated AMPK. The result shows that PD significantly increased the phosphorylation level of AMPK in cells with or without PE stimulation (Fig. 5, A and B), suggesting that PD can suppress the NFAT pathway through activation of AMPK in addition to inhibition of Ca²⁺ signaling.

PD inhibited pressure overload (PO)-induced cardiac hypertrophy in mice. The in vitro data strongly suggest that PD can prevent the development of hypertrophy in vivo through...
the mechanisms indicated above. We thus investigated the effect of PD on PO-induced hypertrophy in mice. Cardiac structure and function were monitored for 13 wk (Fig. 6A). Cardiac structure assessment involved measurement of the thickness of the left ventricular posterior wall (LVPW) and left ventricular internal dimension (LVID). Both systolic (LVPWs) and diastolic LVPW (LVPWd) were increased dramatically at 2 wk of TAC. The LVPWs was increased to a peak at 5 wk of

Fig. 6. PD prevented transverse aortic constriction (TAC)-induced hypertrophy and cardiac dysfunction. A: representative M-mode tracings of echocardiography of mice at 5 and 13 wk of TAC or sham operation, with or without PD treatment. B and C: quantification of the thickness of left ventricular posterior wall at systole (LVPWs, B) and diastole (LVPWd, C). D and E: quantification of systolic left ventricular internal dimension (LVIDs, D) and diastolic left ventricular internal dimension (LVIDd, E). F and G: quantification of left ventricular fraction shortening (LVFS, F) and LV ejection fraction (EF, G). *P < 0.05, **P < 0.01 vs. sham; #P < 0.05, ##P < 0.01 vs. TAC (n = 10 in each group).
TAC, and then decreased gradually. Despite this, the LVPW was still significantly larger than that in sham 13 wk after TAC (Fig. 6B). The LVPWd was increased by 41.5 ± 5.6%, 53.2 ± 6.1%, 50.8 ± 6.5%, and 48.7 ± 6.5% at 2 wk, 5 wk, 8 wk and 13 wk of TAC compared with sham (Fig. 6C). The LVIDs and LVIDd decreased significantly at wk of TAC and then increased gradually and significantly larger than control at 13 wk of TAC (Fig. 6, D and E), suggesting that eccentric hypertrophy developed following the concentric hypertrophy. The hearts were isolated and the heart-to-body weight (HW/BW) ratio was measured at 13 wk of TAC. Figure 7, A and B, shows that the hearts were enlarged and the HW/BW ratio was significantly increased in mice subjected to TAC. There was no significant difference in BW in four groups (data not shown). The protein levels of ANP and β-MHC were accordingly increased in TAC mice (Fig. 7, C and D).

As anticipated, PD treatment largely reversed the hypertrophic remodeling. The values of LVPWs and LVPWd were not significantly changed at 2 to 8 wk of TAC and were only slightly increased at 13 wk of TAC compared with sham (Fig. 6, A–C). Meanwhile, PD treatment ablated TAC-induced alteration of LVIDs and LVIDd (Fig. 6, D and E). The HW/BW ratio and the protein levels of ANP and β-MHC were consistently decreased by PD treatment (Fig. 7). The data indicate that PD prevents the development of PO-induced hypertrophy.

**PD improved PO-induced cardiac dysfunction.** The cardiac systolic function was assessed by left ventricular fractional shortening (LVFS) and LV ejection fraction (EF). The LVFS and EF were significantly increased at 5 wk of TAC, but returned to normal at 8 wk of TAC and significantly lower than normal at 13 wk of TAC (P < 0.01, Fig. 6, F and G). With PD treatment, both EF (14.2 ± 4.4%) and LVFS (16.1 ± 2.5%) were moderately increased at 5 wk of TAC compared with sham (P > 0.05, Fig. 6, F and G). The LVFS and EF were still maintained at relatively high levels at 8 wk of TAC and significantly higher than those without PD treatment (all P < 0.01). Both LVFS and EF were not significantly different from those in sham but significantly higher than those in TAC without PD treatment at 13 wk of TAC (Fig. 6, F and G).

**PD inhibited Ca²⁺-activated calcineurin-NFAT pathway in TAC mice.** The above data suggest that compensatory concentric hypertrophy developed at 5 wk of TAC. We thus measured Ca²⁺ signaling, calcineurin activity, and NFAT nuclear transport at this time point. Consistent with the enhancement of cardiac function (both EF and LVFS were increased), the amplitude of AP-elicited Ca²⁺ transient and myocyte contractility in cardiomyocytes isolated from TAC mice were significantly increased (n = 85 and n = 80 for TAC group and sham group, respectively, P < 0.01 vs. sham). The T₅₀ of Ca²⁺ transient was significantly increased in TAC mice (P < 0.01, Fig. 8D), suggesting the impairment of SR Ca²⁺ cycling in hypertrophic cardiomyocytes. In parallel with the alteration of Ca²⁺ signaling, the calcineurin phosphatase activity was significantly increased and NFAT transferred into nuclear in TAC (P < 0.05, Fig. 8, E–H). PD treatment corrected the abnormal Ca²⁺ signaling, in which both the amplitude and T₅₀ of Ca²⁺ transient were returned to normal (Fig. 8, A, B, and D). The activation of calcineurin-NFAT pathway was concomitantly abolished by PD treatment (Fig. 8, E–H). The results collectively indicate that PD prevented the pathogenesis of PO-induced hypertrophy by inhibition of the Ca²⁺-activated calcineurin signaling pathway.

Of note, the cardiac contractility in isolated ventricular myocytes from PD-treated TAC mice was still significantly
higher than that in sham (Fig. 8C), despite that the amplitude of AP-elicited Ca\textsuperscript{2+} transient was not significantly different from that in sham. The increase in cellular contractility in single cardiomyocyte is consistent with the increase of LVFS and EF in PD-treated TAC mice (Fig. 6, F and G).

**DISCUSSION**

In this study, we monitored the effects of sustained long-term PO on cardiac structure and function and demonstrated a typical development of the heart from “compensatory” cardiac hypertrophy to “decompensatory” heart failure. An important
finding in this study was that PD has a potent therapeutic effect on preventing the development of cardiac hypertrophy and deterioration to heart failure upon sustained PO stimulation. Furthermore, we found that PD dose-dependently prevented hypertrophic remodeling in cultured cardiomyocytes in vitro. The results indicate that PD can target directly on cardiomyocytes to prevent the pathogenesis of hypertrophy. It is well established that Ca\(^{2+}\)-regulated signaling pathways contribute to the pathogenesis of cardiac hypertrophy. For gene transcription, the Ca\(^{2+}\)/calmodulin (CaM)-activated calcineurin-NFAT signaling pathway has been shown to play a major role in the development of pathological cardiac hypertrophy. Upon dephosphorylation by calcineurin, NFAT translocates to the nucleus of the cardiomyocyte where it mediates the transcription of numerous targets involved in hypertrophic growth (27, 32). In this study, we found that calcineurin activity was upregulated and the nuclear NFAT protein level was increased in both PE-induced hypertrophic cardiomyocytes and in PO-induced hypertrophic heart. In accordance with the activation of the calcineurin-NFAT signaling pathway, the amplitude of systolic Ca\(^{2+}\) transient in cardiomyocytes was significantly increased. Meanwhile, the SR Ca\(^{2+}\) recycling indicated by T\(_{50}\) of Ca\(^{2+}\) transient was slowed, indicating the disturbance of intracellular Ca\(^{2+}\) homeostasis. Of importance, blockade of LTCC with nifedipine or inhibition of calcineurin activity with FK506 significantly suppressed PE-induced hypertrophic remodeling. This in vitro and in vivo evidence is in support of the key role of the Ca\(^{2+}\)-activated calcineurin-NFAT signaling pathway in the development of cardiac hypertrophy.

Since upregulation of Ca\(^{2+}\) signaling increases cardiac contractile function, which represents “compensatory” response projected to normalize wall stress and facilitates systolic performance, inhibition of Ca\(^{2+}\) signaling with a LTCC blocker suppresses cardiac function and is not beneficial for the treatment of cardiac hypertrophy. In this study, we found that PD inhibited the calcineurin-NFAT signaling pathway. PD attenuated the development of cardiac hypertrophy both in vitro and in vivo. Furthermore, we found that this effect was mediated by inhibition of cardiac Ca\(^{2+}\) handling, for PD completely abolished the increase of the amplitude of systolic Ca\(^{2+}\) transient in cultured cardiomyocytes upon PE stimulation and in single cardiomyocytes isolated from hearts challenged with PO. Of particular interest, PD, unlike Ca\(^{2+}\)-channel blockers, did not impair cardiac contractile function, despite that PD significantly decreased the amplitude of systolic Ca\(^{2+}\) transients. Moreover, PD restored the impaired SR Ca\(^{2+}\) recycling, which has been well documented, inducing cardiac dysfunction in heart failure by causing Ca\(^{2+}\) overload during diastole. The unique regulations of PD on Ca\(^{2+}\) handling and contractility in cardiomyocytes indicate that PD is superior to Ca\(^{2+}\)-channel blockers on the treatment of cardiac hypertrophy.

Previous study has demonstrated that PD can also inhibit calcineurin activity. However, the causal link between the modulations of Ca\(^{2+}\) signaling and calcineurin activity remains unknown. This present study reveals for the first time that PD modulation of intracellular Ca\(^{2+}\) signaling is an important mechanism for PD inhibition of the activation of calcineurin-NFAT pathway, which may also be the case for the action of resveratrol. The ability of PD to inhibit Ca\(^{2+}\) signaling and subsequent calcineurin-NFAT signaling pathway may represent its pivotal effect in terms of attenuating the hypertrophic response. However, other mechanisms should also participate in the antihypertrophic effect of PD. In this study, we found that PD significantly increased AMPK activity in PE-stimulated cells, suggesting the involvement of this signal pathway in PD suppression of cardiac hypertrophy.

In conclusion, our present study demonstrated that PD had a potent antihypertrophic effect in PE-induced cardiomyocyte hypertrophy and PO-induced cardiac hypertrophy. Furthermore, PD prevented the development of cardiac contractile dysfunction under sustained PO. The beneficial effects of PD were largely attributed to its modulation of Ca\(^{2+}\) handling and inhibition of subsequent activation of the calcineurin-NFAT signaling pathway without compromising cardiac contractile function.

ACKNOWLEDGMENTS
We thank Huaiwang for kindly providing us polydatin.

GRANTS
This work was supported by the National Science Foundation of China (Nos. 31171096, 31371159, and 81200122) and the Basic Research Foundation of SZ (JC201005250059A, JCY2012061311553598, GJHS20120621143653775, and GHS2012062114367572).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: W.D. and M.D. performed experiments; M.D. analyzed data; J.D. and T.X. interpreted results of experiments; D.Y. approved the final version of manuscript; Y.L. and J.L. prepared figures.

REFERENCES


AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00017.2014 • www.ajpheart.org